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CORRELATION OF TISSUE MUCOPOLYSACCHARIDES WITH THE HAIR CYCLE*

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The purpose of this report is to demonstrate in the skin of rats the fluctuations of heparin and other acid mucopolysaccharides (AMPS) during an induced hair cycle.

MATERIALS AND METHODS

Four groups of 4 to 5 Wistar S.M. male albino rats each were killed on days 2, 7, 13, 18, 21, 25, and 30 of the 3rd hair cycle (G3(1)) which had been induced by plucking.

The whole skin of the back was shaved, excised to the depth of the panniculus carnosus and freed of all visible adherent fat, and roughly subdivided.

Since large amounts of material are required for these determinations, the skin of 4–5 animals was pooled and treated with a slightly modified Schiller's technic (2) for extraction of AMPS and separation of their fractions.

The basic steps of this lengthy procedure, lasting about 45 days, can be thus summarized:

1. Drying and thorough de-fatting by Soxhlet apparatus.
2. Complete digestion of material with papain; dialysis in running tap water; dialysis in distilled water; digestion with trypsin, dialysis in phosphate buffer at pH 7.8; precipitation with trichloroacetic acid.
3. Centrifugation; further dialysis in distilled water; concentration of AMPS.
4. Precipitation of AMPS by cetylpyridinium chloride (CPC); separation of the fractions by different NaCl molar solutions: 0.4 M in 0.1 per cent CPC for hyaluronic acid; 1.2 M in 0.1 per cent CPC for chondroitinsulfuric acid; 2.1 M in water for heparin.
5. Colorimetric quantification of each fraction with carbazole (3). On each of the designated days, three different evaluations of both total AMPS and their single fractions were made from 4 different pools.

All data were submitted to the evaluation of the SEM and the confidence interval and to the analysis of variance (4).

RESULTS

1. The total content of AMPS (Fig. 1, Table I) varied greatly during each cycle. It increased during the first week of anagen from slightly more than 1700 gamma/g.d.w. to about 2000 gamma, and steadily decreased throughout the rest of the cycle, between the 7th and 21st day, to values around 1100 gamma at the end of telogen.

2. Of the individual AMPS fractions, the largest was hyaluronic acid (Fig. 2, Table I) which gradually fell from about 1000 gamma at the beginning of anagen to about 600 gamma at the end of telogen.

Meanwhile, chondroitinsulfuric acid (Fig. 3, Table I) increased from values of 600 gamma to values approximately 750 gamma until the 7th day of anagen when it rapidly decreased in the following week to less than 500 gamma; it continued to drop slowly to values around 400 gamma at the 18th day and remained practically steady in catagen and telogen. Heparin (Fig. 5, Table I) rose sharply in the first days of anagen from about 90 to 160 gamma and then fell just as quickly after the 7th day to the previous values (90 gamma at the 13th day).

During the entire cycle, the loss of heparin was proportionally greater (about 70%) than that of the other two fractions of AMPS (about 40%) (Fig. 5). At the end of anagen, heparin values were 50 gamma, *i.e.*, less than a third than at the peak of the curve.

Scarcely any fluctuation occurred during the rest of the cycle, though the wide confidence limits in this and in other fractions may indicate a slight rise after the 25th day.

The total recovery was 94% ± 2 with no significant difference between the days of cycle.

All AMPS fractions, then, behaved alike: the high content on days of new hair formation dropped more or less steeply in the periods of hair differentiation, transition, and rest. These fluctuations are statistically highly sig-

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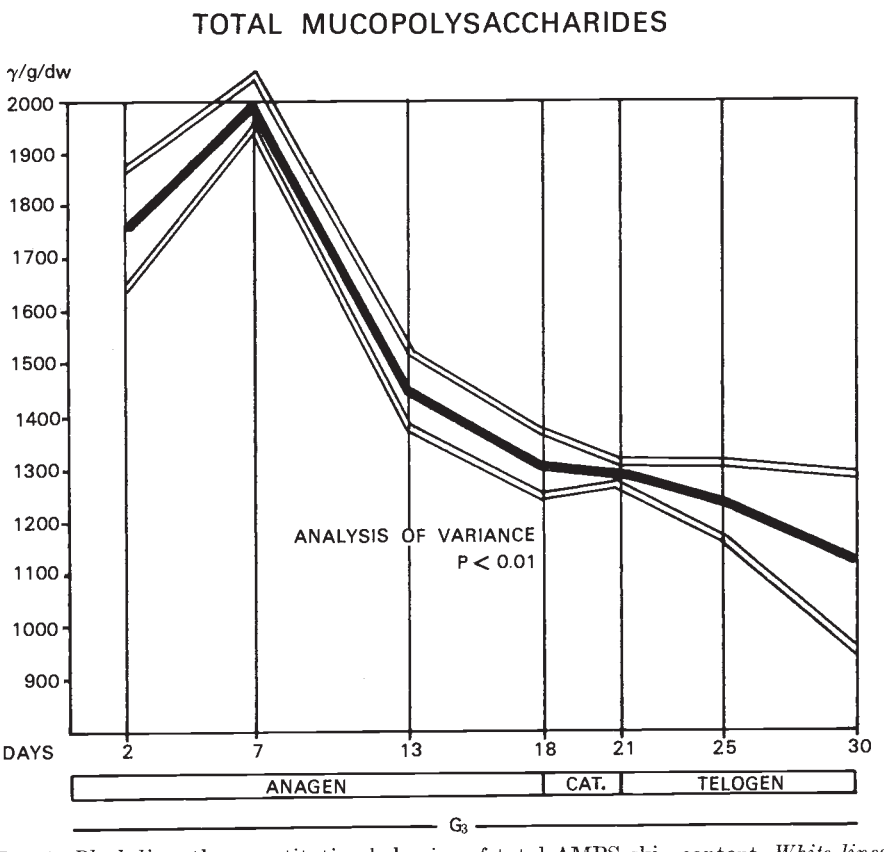

FIG. 1. *Black line:* the quantitative behavior of total AMPS skin content. *White lines:* the confidence limits.

TABLE I

Days of life (G3)	Experimental days	Hair cycle phases	Number of pools	Total AMPS gamma/g.d.w. ± confid. interv.	Hyaluronic acid gamma/g.d.w ± confid. interv.	Chondroitin sulf. acid. gamma/g.d.w. ± confid. interv.	Heparin gamma/g.d.w. ± confid. interv.
66	2	Anagen	4	1764 ± 101	1033 ± 88	609 ± 26	91 ± 11
71	7	Anagen	4	1988 ± 52	1012 ± 17	739 ± 54	160 ± 14
77	13	Anagen	4	1447 ± 86	795 ± 11	478 ± 102	91 ± 18
82	18	Catagen	4	1319 ± 81	782 ± 66	416 ± 56	50 ± 14
85	21	Catagen	4	1308 ± 15	730 ± 40	420 ± 34	50 ± 7
89	25	Telogen	4	1265 ± 74	674 ± 170	425 ± 72	41 ± 16
94	30	Telogen	4	1138 ± 171	599 ± 122	432 ± 41	48 ± 25
Analysis of variance				F = 105.9 when F 0.01 = 3.7	F = 27.0 when F 0.01 = 3.7	F = 42.8 when F 0.01 = 3.7	F = 70.2 when F 0.01 = 3.7

nificant (Table I). The greatest shifts of AMPS material were observed in heparin.

CONCLUSIONS AND COMMENTS

Our AMPS evaluations in rats of the same age were higher than those of Schiller (5), who employed the less sensitive colorimetric method of Dische (6).

All curves obtained were clearly parallel to those of the mast cell (7–9).* For hyaluronic

* Previous mast cell counts were performed,

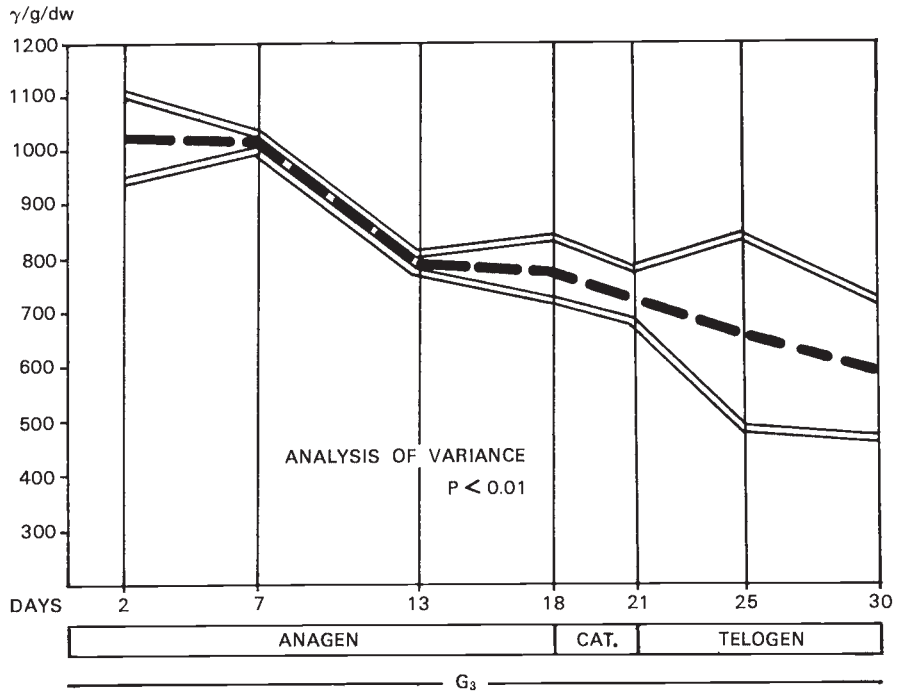

FIG. 2. *Black line:* the quantitative behavior of hyaluronic acid skin content. *White lines:* the confidence limits.

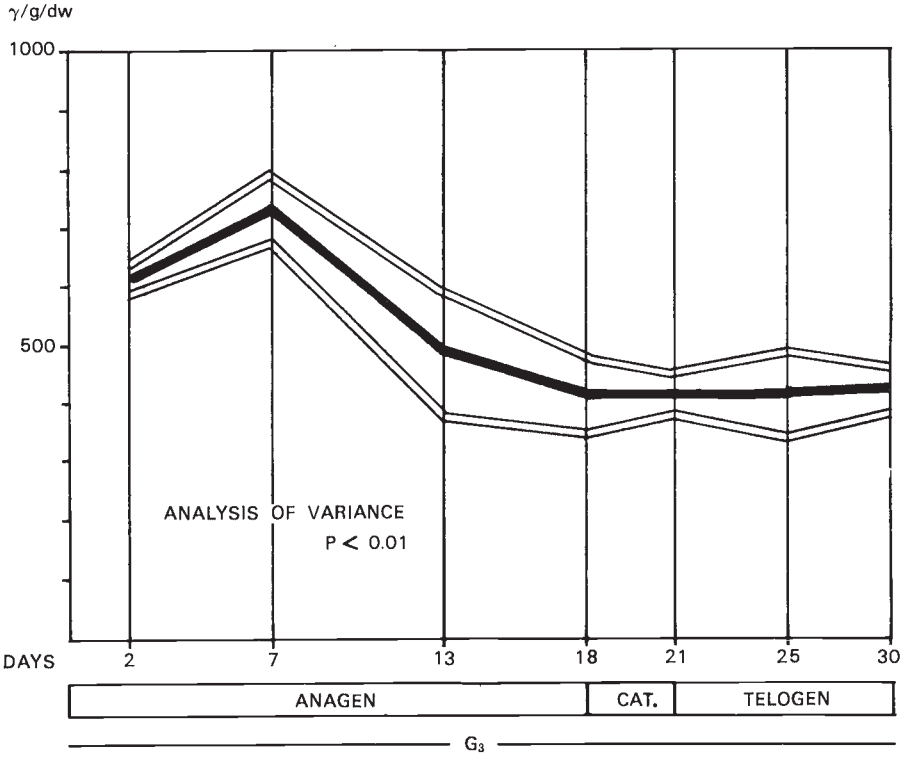

FIG. 3. *Black line:* the quantitative behavior of chondroitinsulfuric acid skin content. *White lines:* the confidence limits.

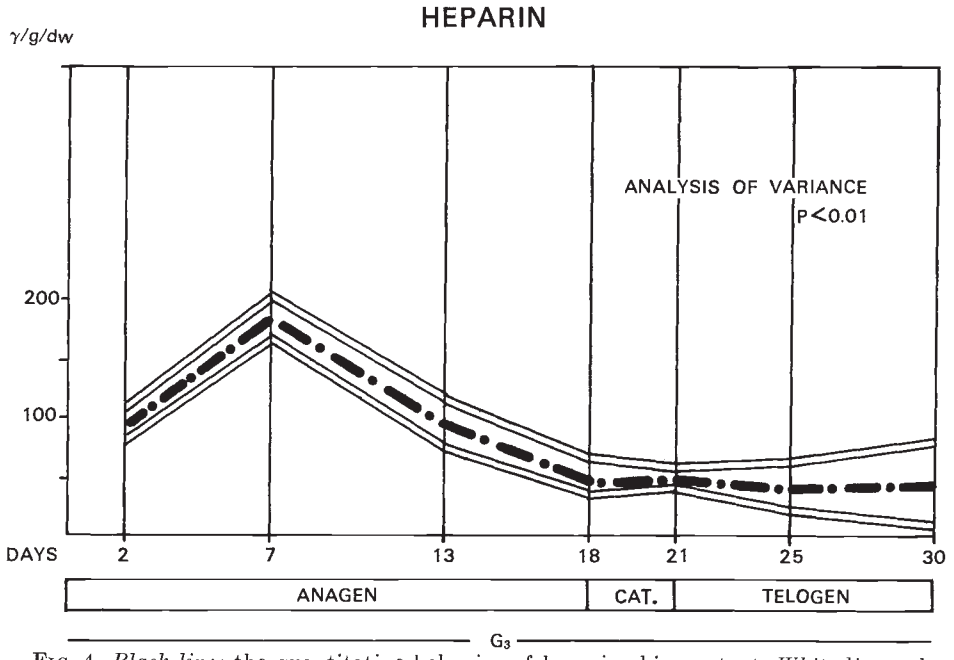

FIG. 4. *Black line:* the quantitative behavior of heparin skin content. *White lines:* the confidence limits.

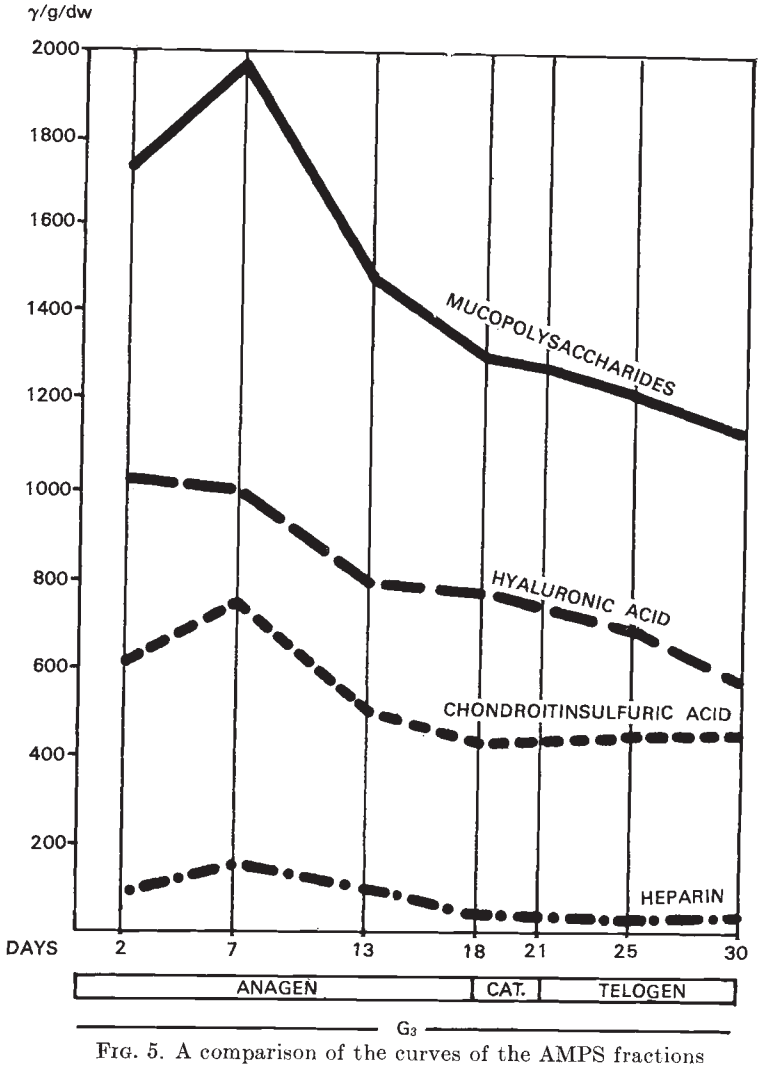

FIG. 5. A comparison of the curves of the AMPS fractions

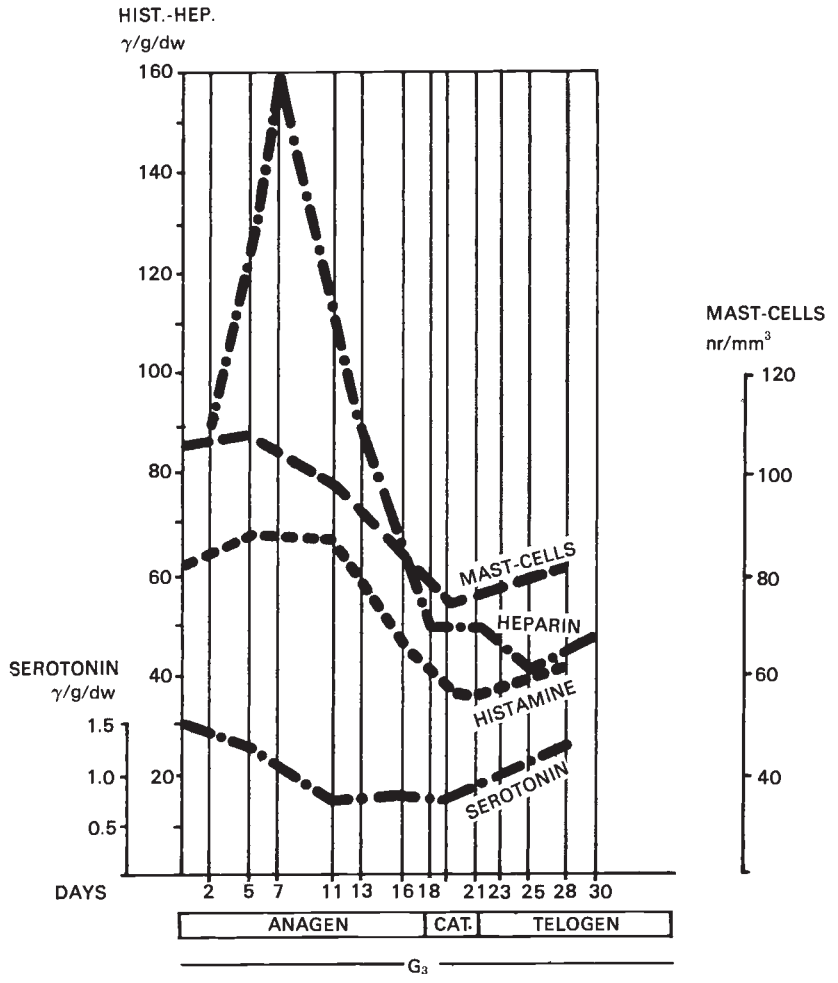

FIG. 6. A comparison of the curve of heparin with those of histamine, serotonin, and mast cells.

and chondroitinsulfuric acid especially, this may indicate a much closer connection with those cells (10, 11) than was previously admitted (12–14), whereas the parallelism with heparin, as well as with the other mast cell metabolite, histamine, was to be expected (Fig. 6) (7–9, 15, 16).

The considerably higher and sharper fluctuations of heparin suggest its predominant role, while the generally elevated levels of AMPS fractions at the beginning of anagen are in agreement with the histochemical changes (17, 18).

Since the concentration of AMPS in the skin was minimal (0.2% at most), it may not appreciably influence its water content. Hence, the fluctuations of water (12–15%) mentioned in the literature (19, 20) may be due, at least in part, to other factors, such as vascularization.

The rise of heparin during the first days of anagen helps to maintain normal blood fluidity in the simultaneously increased perifollicular blood networks (21) which are dilated and more permeable because of histamine (8).

Heparin and other AMPS may also influence normal multiplication and differentiation of hair cells as a labile sulfur donor supporting keratin synthesis in anagen (17) and initiating a new hair cycle (23) when injected intradermally into rabbits.

however, within considerably smaller skin fraction than those considered here (7–9).

These findings obviously contrast with the well-known hair loss provoked by heparin sodium and heparinoids (22, 24).

SUMMARY

The considerable quantitative fluctuations of hyaluronic acid, chondroitinsulfuric acid, and heparin in the skin of rats are apparently related to the stages of the hair cycle.

All fractions of acid mucopolysaccharides behave similarly: high on days of new hair formation, they drop in hair differentiation, transition, and rest. The greatest shifts (70%) in AMPS material, however, are seen in heparin during the first week of anagen.

These curves clearly parallel those of the mast cell and histamine.

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